

In this study, it is important to detect the time of occurrence of change in phase of activity of Spm and to derive progeny from a cell in which this occurred. For this reason, the majority of tests of Spm behavior were conducted with ears of plants rather than with pollen because cell lineages are more evident in them. Each plant had a limited number of fertile ears that could be used for test purposes and a decision had to be made in the test of any one plant regarding the type of tester plant that should be used as pollen parent in the cross to each ear of the plants whose Spm was desired to be examined. If the appearance of the stalk and leaves of the plant indicated that it had started its development with an active Spm, a cross was usually made with it using pollen of a plant having no Spm that was homozygous for a_2 and bt, and either Wx or wx, depending on the constitution of the ear-bearing plant. If the appearance of the plant indicated that Spm was in its inactive phase in most parts of the plant, then a cross using pollen of a plant homozygous for a_2 , bt, and carrying an active Spm was indicated for at least one of the fertile ears of the plant, and for reasons that will be made evident shortly. When possible, another ear of this same plant was used in a cross with a plant homozygous for a_2 and bt and having no Spm. Ears of some plants having either an initially active Spm or an initially inactive Spm in them

were used in crosses with plants that carried a class I state of a_2^{m-1} and bt, but in which no Spm was present, in order to examine the response of the chosen class I state of Spm that was contributed by the ear-bearing parent. Since many of the tested plants were a_2^{m-1} (class II) Bt/ a_2 bt in constitution, the direct response of the class I state to Spm in the female parent could be observed among the bt class of kernels on the resulting ear, as the large majority of them receive a_2 and bt from the ear-bearing parent.

In the summer of 1957, the only plants homozygous for a_2 , bt, and wx and having an active Spm in them were plants 7308D-1 and D-2. The origin of these two plants from the a_2 , bt, wx class of kernels on the self-pollinated ear of tiller-1 of plant 7109B-2 was described earlier. Both plants produced three tillers. Pollen collected from the tassel of the main stalk and from that of each tiller was used in making crosses to a large number of plants having either a class I or ~~xxxxxx~~ the class II state of a_2^{m-1} and carrying either no Spm or one or more Spm elements in the active or the inactive phase in different parts of the plant. The phenotypes of kernels on the ears these crosses produced indicated that plants 7308D-1 and D-2 each had one Spm and that in the pollen derived from all parts of each plant, the Spm was in its active phase in nearly all of the pollen

grains that carried it. From the phenotypes of the kernels on some of these ears, it was first learned that it was possible to distinguish whether or not a uniformly pigmented, ear-bearing plant had no Spm in it or had an Spm that was in its inactive phase. This is because Spm in its inactive phase, although unable ~~alone~~ to effect suppression of gene action with the class I and class II states of a_2^{m-1} , and to induce mutation with the class I states, behaves as if it were in its active phase when it is in the same nucleus with an Spm that is in its active phase. However, this association does not induce an alteration in the phase of activity of either Spm. When the active and inactive Spm elements are segregated into different nuclei by the meiotic process, the active Spm element continues in its active phase whereas the previously inactive Spm element reappears in its inactive phase. This type of test is so effective that it has been used extensively in succeeding years to determine whether or not an inactive Spm is present in a part of a plant that exhibits no evidence of the presence of Spm by the phenotype of its stalk, leaves, or tassel. Use of this test has made it possible to insert the "-" symbol in many of the places where it appears in summary tables 2, 3 and 4. Illustrations may now be given of the types of test that were conducted with each plant in a culture and the effectiveness of each test in determining the presence

or absence of Spm in a plant and its phase of activity in the various tested parts of a plant, should Spm be present in it. For this purpose, tests of the progeny derived from the ear of the main stalk of plant 7109B-1 (see tables 1 and 2) and from the ear of the main stalk of plant 7109C-4 (see tables 1 and 4) may be described first.

Kernels were selected from the first ear of the main stalk of plant 7109B-1, which was a_2^{m-1} (class II) Bt/ a_2 bt, Wx +/wx Spm in constitution, and the plants grown from them in the summer of 1957 received culture number 7456. The plants in A, B, and E of this culture were derived from uniformly pigmented kernels, those in A and B from the Bt class and those in E from the bt class. Plants derived from Bt kernels exhibiting the "diffuse-mottled" phenotype were grown under C of culture

7456, and those derived from Bt kernels that ~~xxxxxxxxxxxxxxxx~~ showed small spots or specks of pigment in a non-pigmented background were grown under D of culture 7456. The types of test cross conducted with 22 of the plants in this culture are entered in table 5. In table 2A, under Year 1957, is recorded the behavior of Spm in the cells giving rise to the tested ears in each of the Spm carrying plants in culture 7456. For each ear, the given symbol referring to activity phase of Spm was based upon the phenotypes of kernels appearing on the ears derived from the crosses entered in table 5.

TAB 6 In table 6 are given the types of test cross that were conducted with plants in culture 7455 that were derived from selected kernels on the first ear of the main stalk of plant 71090-4. This plant was Wx/wx and carried 1 Spm that was not linked to either of these alleles. The plants in A, B, and D of culture 7455 were derived from Bt kernels that exhibited the "diffuse-mottled" phenotype. Those in C of culture 7455 were derived from Bt kernels that had spots or specks of color in a colorless background. As table 4 indicates, each of the 18 tested plants in culture 7455 had an Spm element in it, and it was in its inactive phase in the cells that gave rise to all tested ears except that of the tiller of plant 74550-4.

Before considering the phenotypes of the kernels on the ears

entered in tables 5 and 6, the e produced from testcrosses types 1, 2,

and 3 ¹ to a plant of the constitution a_2^{m-1} (class II) $Bt/a_2^{m-1} Bt$; ^(class I)

$Wx +/wx$ Spm (active), will be considered, as the data obtained from crosses

with it may be compared with those obtained from the crosses of plants

entered in tables 5 and 6. This plant, 7308A-4, was derived from a tiller-2 of

kernel on the self-pollinated ear of plant 7109B-2, described on page .

The appearance of this plant ^{7308A-4} indicated that it had started development with an sp in its active phase.

The phenotypes of the kernels on the three tested ears of this plant from

use of pollen ¹ tester plants types 1, 2, and 3, are given in table 7.

kernels of the first ear of the main stalk received pollen from plant

7308D-2 (type-3 tester) that was $a_2 bt/a_2 bt$, $wx +/wx$ Spm (active) in

constitution. On this ear, a 1 : 3 ratio of uniformly pigmented kernels

to colorless kernels with spots or specks of pigment ¹ among the latter,

there were three distinguishable classes with respect to number and size

of pigmented areas: those that exhibited a numerous pigmented spots, ~~a~~

number of which covered a large area ("1 Spm pattern"), those that

exhibited a number of pigmented ^{spots} ~~areas~~, all of which were small in area

("2 Spm pattern") and those that exhibited only small specks of pigment,

and often very few of them per kernel ("High dose Spm pattern"). The

distribution of wx and wx among the 4 classes of kernels on this ear

indicated that plant 7308A-4 carried an ~~active~~ Spm that was ~~closely~~ linked ^{and that it was in its active phase in the cells that gave rise to this ear} with wx. [^] It also demonstrates the effect of dose of Spm on pattern of

pigmented spots. The class with the "1 Spm" pattern received its Spm from the male parent. The class with the "2 Spm" pattern received its Spm ^{elements}

from the female parent, as the female contributes two haploid nuclei to the

^{primary} endosperm. ^{nucleus} The class with the "high dose Spm" pattern received two Spm

elements from the female and ^{one} Spm element from the male. Within each

of these classes there could be a few misclassifications with regard to ^{this suggested change} ~~parental~~

origin of Spm as ~~the~~ cross ~~types~~ types 1 and 2 will illustrate.

Cross type-2, conducted with the second ear on the main stalk of plant 7308A-4, again showed the presence of an active Spm ^{in the cells that gave rise to this ear and} ~~in this plant~~ that ^{was} ~~was~~ [^] ~~closely~~ linked with wx. Among the 129 variegated kernels on this ear,

two exhibited the "high dose Spm" pattern. Tests of plants arising from

kernels of this type on ears of plants having one Spm have demonstrated

that ^{usually} they carry more than ^{one} Spm in them. The origin of the extra Spm may be attributed to ^{the} transposition mechanism.

The tiller ear of plant ~~73~~ 08A-4 was used in a cross with tester type-1. A ratio of 1 uniformly pigmented : 1 variegated kernel appeared on this ear. Among the variegated kernels, one exhibited the "1 Spm" pattern. It is quite probable that the cells of the endosperm of this kernel have

only 1 Spm in them because only one of the two nuclei delivered by the female gametophyte ^{to the primary endosperm nucleus} carried an Spm. The other nucleus ^{could have} ~~may have~~ lost Spm through the transposition mechanism during gametophytic development.

If a plant has no Spm in it, ^{all parts of the plant are pigmented, and also,} then with testcross types 1, and 2, all a_2^{m-1} (class II) carrying kernels are uniformly dark pigmented. No variegated kernels appear on these ears. If, however, the tester plant is of type 3, then ^{approximately} half of the a_2^{m-1} kernels are uniformly dark pigmented and half are variegated: colorless with spots of pigment in them. The phenotype of the kernels on ears of "no Spm" plants in culture 7456 (table 5) and of ^{such} 4 plants in culture 7308A, when crossed with tester plants of type 3

^{7308A} (that is, plants 7308D-1 or D-2) are given in A of table 8. On the ears of the two plants (7308A-1 and A-2) that were a_2^{m-1} Bt/ a_2^{m-1} Bt there were 432 uniformly dark colored kernels and 404 kernels that showed colored areas in a colorless background. Among the latter, 402 exhibited many pigmented spots, some of which were large in area. ^(1 Spm pattern) Within these large pigmented areas, smaller colorless spots were present (see figure).

Two of the variegated kernels exhibited only small spots ~~or specks~~ of pigment. ^(2 Spm pattern) Among the 953 a_2^{m-1} Bt carrying kernels derived from the cross of the a_2^{m-1} Bt/ a_2 bt plants, ^{to the tester type-3 plants in A of table 8,} 475 were uniformly dark pigmented and 478 were colorless with spots of pigment in them. In 468 of these latter,

there were many pigmented areas, a number of which were large (1 μ m pattern) and in the remaining 10 variegated kernels, there were fewer pigmented areas and all of them were small ~~in area~~ (more than 1 μ m). Among the total of 1789 a_2^{m-1} Bt kernels in table 8, 907 were uniformly pigmented and 882 were variegated; 870 of the variegated kernels exhibited the "1 μ m" pattern and 12, or 1.35%, exhibited a pattern expressed when more than one μ m is present in the endosperm. It is evident that among the μ m carrying pollen grains produced by plants 7308D-1 and D-2, ^(the tester type-3 plants) the vast majority ~~had~~ only one μ m in each. It is also ~~clear~~ ^{evident} that ⁱⁿ nearly half of the pollen grains ~~these plants produced had an active μ m in them.~~ ^{an μ m in its active phase was present.} Among a total of 5040 pollen grains that could be tested for this in crosses to 28 plants that had no μ m in them, 2577 ~~pollen grains~~ gave no evidence of μ m in any one of them whereas the remaining 2463 pollen grains ^{carried} ~~had an~~ active μ m ^{and among them} ~~in them~~ of which approximately 98 percent had but one μ m. The remaining 2 percent had more than 1 μ m in each.

The types of kernels appearing on the ears of plants in culture 7455 (table 6), when crossed by tester plants of type-3 (plants 7308D-1 and D-2), are entered in B of table 8. Among the a_2^{m-1} carrying kernels on these ears, an approximate 1 : 1 ratio was given of uniformly pigmented kernels to those that had pigmented spots or specks in a colorless background.

The latter kernels could readily be distributed, on the basis of pattern of pigmented spots, into two main classes: those that exhibited a number of spots of pigment, some of which were large in area (the "1 Spm" pattern) and those that exhibited a pattern that is known to appear when more than 1 Spm is present (see figures). The ratio of these two classes ~~closely~~ approximated 1 : 1 on the majority of the ears, as shown in the table.

That Spm was present in these plants but in its inactive phase in most parts of the plant was made evident by the appearance of sectors, usually ^(usually small in area, in the stalk, leaves and tassels) found only in the tillers. ^{these sectors, was in} In ~~which~~ Spm had ~~changed from its inactive to its active~~ phase. These sectors were ^{entirely} non-pigmented or non-pigmented with some small streaks ^{within them} in which pigment was present.

Among the ears of plants in culture 7455 that were used in making testcross types 1 and 2 (see table 6), there was no evidence of the presence of Spm among the kernels of 6 of the 14 ears. Its presence was made evident in some kernels on the ~~xxxxxxxxxxxx~~ remaining 8 ears (table 9).

Only in the cells of the tiller ~~ear~~ ^{that produced the ear} of plant 7455C-4 was Spm in its active phase. ^{the cells produced} In all other ears, ^{Spm was in its inactive phase, but it had} ~~Spm~~ entered the active phase ^{and was changed to} only in some cells late in development of the ear or ^{in some cells} during development of the kernel. ^{never}

The symbols describing the behavior of Spm in different parts of the plants in culture 7455, placed in table 4 (year 1957), are based on the

phenotypes of the kernels derived from the test crosses described above.

That the designations of Spm phase in this table are based on correct

interpretations of the origins of the phenotypes on the ~~described testcrosses~~

produced from the described test crosses,

ears ¹ comes from similar types of test conducted with the plants in culture

7456 (table 5). In the crosses conducted with plants in culture 7455, no

evidence was given of linkage of the inactive Spm with ~~the~~ ^{wt} in those plants

that were wx/wx in constitution. However, such evidence was given by the

wx/wx , Spm carrying plants in culture 7456. The parent plant (7109B-1)

was known to be $\text{wx} +/\text{wx}$ Spm in constitution and ^{the majority} ~~many~~ of the wx/wx plants

in culture 7456 should ^{also} ~~also~~ be $\text{wx} +/\text{wx}$ Spm in constitution. The appearance

of the kernel from which each plant in culture 7456 arose was described

on page . Twelve tested plants were derived from uniformly dark

^{pigmented} ~~colored~~ kernels (those in A, B, and E of this culture). Nine of these

twelve plants were wx/wx and in none of them was any evidence given,

either by the appearance of the plant or by the appearance of the kernels

derived from test crosses with each, of the presence of Spm in them.

^{phase} The types of kernels appearing on the ears of these plants, ^{produced by} ~~following a~~

^{test cross} ~~cross with tester type-3~~, ^{was} discussed earlier, and the data obtained from

each such cross ^{is} ~~was~~ entered in A of table 8. Among the 12 ears ^{we} obtained

from testcross types 1 ^{or} ~~and~~ 2, of the ^{parent} $\text{a}_2^{\text{m-1}}$ Bt/ a_2bt , wx/wx plants, ^{Among the kernels on these ears,} no

evidence whatsoever was given of the presence of α pm in any one of them.

There was a total of 3107 kernels; 1484 were uniformly dark pigmented (1415 Bt : 69 bt) and 1533 were totally colorless (79 Bt : 1454 bt).

That the e plants carried the class II state of a_2^{m-1} that was capable of responding to the presence of an active β pm was shown by testcross type-3 conducted with 6 of these 7 plants (A, table 8). The remaining 3 of the 12 tested plants that arose from the uniformly dark pigmented kernels were Wx/wx . No evidence of the presence of β pm was given by plant 7456B-6, either by the appearance of the plant or by testcross type-1 conducted with the one fertile ear it produced. Both of the other two plants (B-5 and E-2) were $Wx +/wx$ β pm. All 4 of the plants in 7456C and all 6 of the plants in 7456D carried β pm. Eight of these 10 plants were $Wx +/wx$ β pm in constitution and 2 were Wx/wx . Both of the latter 2 plants had one β pm. It is probable that the β pm in each of them was located close to Wx in one chromosome 9 but progeny tests would be required to determine this, and these were not conducted.

Testcross type-3 was conducted with an ear of 5 of the 10 ~~xxxx/xxxxxxx~~ Wx/wx plants in culture 7456 and with each of the 2 ^{plants} that were Wx/wx and carried α pm (plants C-1 and D-4). In table 10 ^A is given the phenotypes of the kernels on ears produced by the 5 $a_2^{m-1} Bt/a_2 bt$, Wx/wx plants

that were subjected to testcross type-3, and in B of table 10 is given the phenotypes of kernels on the ears of the two Wx/Wx plants carrying Spm that were subjected to this test. On each ear in A of table 10, approximately half of the a_2^{m-1} carrying kernels were fully pigmented and half were variegated in that they showed either many spots of pigment in a colorless background ("1 Spm" pattern) or a few small spots or specks of pigment in a colorless background ("high dose" Spm pattern). The distribution of Wx and wx among these two classes of variegated kernels is strikingly unequal. The great majority of the Wx kernels exhibited the "1 Spm" pattern of pigmented spots whereas the great majority of wx kernels exhibited the "high dose" Spm pattern. It will also be noted that the majority of kernels exhibiting the "diffuse-mottled" phenotype were wx (2 Wx to 17 wx). If the latter kernels are combined with the uniformly pigmented class of kernels, the ~~ratio of~~ ^{frequency of kernel} types would be: 207 uniformly pigmented, Wx, ~~xxxxxx~~, 206 uniformly pigmented wx, 200 variegated kernels exhibiting the "1 Spm" pattern of spots (178 Wx to 22 wx) and 209 kernels exhibiting the "high dose" Spm pattern of pigmented spots (24 Wx : 185 wx). It is quite evident that a factor ~~closely~~ ^{intimately} linked with wx (11 crossover units) in these plants is responsible for the "high dose" Spm pattern of pigmented spots in those kernels that received an active Spm from the type-3 tester

pollen parent. That this is Spm in its inactive phase in the cells that gave rise to these ears could be suspected as the parent plant, 7109B-1, was $Wx +/wx$ Spm in constitution and Spm was ~~closely~~ linked with wx . Also, the ^(recombination) ~~crossover~~ percent ^{this inactive} between wx and Spm is similar to that given by plants derived from the tillers of plants 7109B-1 and B-2 that were $Wx +/wx$ Spm (active) and illustrated ^{see earlier} in tests conducted with plant 7308A-4, entered in table 7. That this factor is Spm ^{also} is shown by the phenotypes of the kernels on ears of these and other Wx/wx plants in culture 7456 that were produced from testcross type-2. The types of kernels on the ears this cross produced are entered in table 11. It will be noted in this table as well as in table 12 (from testcross type-1) that the kernels on ears produced by the main stalk of these plants gave either no evidence of Spm in any one of them or its presence was made evident in only a very few kernels on an ear. On the ears produced by the tillers, many more kernels showed the presence of Spm in them and the number of them ^{etc} increased progressively, the later the emergence of the tiller from the base of the plant. In other words, the younger tillers had more such kernels. Among the kernels exhibiting Spm , linkage of it with wx was clearly expressed, and it could be located approximately 10 ^{distinct} crossover units from wx .

The evidence obtained from the testcrosses described above makes it obvious that union in the primary endosperm nucleus of two nuclei contributed by the female gametophyte, in each of which an inactive Spm is present, with a nucleus contributed by the male gametophyte in which Spm is active, will give rise to a kernel whose aleurone layer will exhibit a pattern of pigmented specks in a colorless background that mimics the pattern produced when 3 active Spm elements are introduced into the primary endosperm nucleus. As indicated earlier in this report, the inactive Spm under these circumstances, does not undergo that particular type of genetic alteration which is responsible for a true change of its phase of activity. It expresses, following meiotic segregation from the active Spm, the same inactive phase that it previously had expressed before being associated with the active Spm. At present, there is no evidence to indicate the level of ~~expression of the~~ ^{whereby which} complementation ^{occurs} ~~that~~

^{between} an inactive and an active Spm, ~~exert on one another~~. However, it is evident that this must occur at some level in these cells, and the fact that ^{it does} ~~this~~ ~~occurs~~ has made it possible to test for the presence of an inactive Spm in parts of plants that otherwise ~~would~~ give no evidence of the presence in them of any Spm element. Tables 13 and 14 summarize the effectiveness of this test, conducted with the test type-3 plants (7302 D-1 ^{and} D-2), obtained from crosses made with these plants during the summer of 1957.

The tests considered so far have dealt with the behavior of Spm in subsequent generations following two cases of change in phase of its activity from active to inactive-- and registered originally in the main stalk of plant 7109B-1 and of plant 7109C-4. The subsequent behavior of the inactive Spm in the second and third generation progeny, is summarized in A of table 2 for that originally present in the main stalk of plant 7109B-1 and in table 4 for that originally present in the main stalk of plant 7109C-4. The origin of the plants entered in these tables is

indicated at the base of each ^{Table, along with} ~~as well as~~ the appearance of the kernel from ^{derived from the main} which each plant arose. It may be seen that the Spm ~~in both cases~~

stalk of each of these plants

continued in its inactive phase in subsequent generations and that return

of it to the active phase was ^{infrequent,} ~~much delayed in the progeny plants,~~ the event responsible for this occurring only in ^{a few} ~~some~~ cells, and often restricted to those of the tillers.

Table 2

The behavior of Spm ^{in the} ~~derived from~~ tiller-1 and tiller-2 of plant

7109B-1 contrasted greatly with ^{to} ~~the~~ behavior of this same Spm that was

~~present in kernels on the ear of the main stalk of this plant,~~ ⁱⁿ ~~and this appears~~ continued to be expressed in subsequent generations

Comparison of A with B and C of table 2 will make this evident. It should

be stated here that pollen collected from the same plant was used in making

crosses to each of the ears of plant 7109B-1 and thus, the difference

in behavior of Spm in the progeny derived from kernels on the ears of this plant cannot be attributed to some modifying factors introduced by the male parent. In the Spm carrying progeny derived from kernels on the ear of the main stalk of plant 7109B-1, Spm was in its inactive phase in the cells that gave rise to ears in all tested parts of each plant and remained in this phase in successive generations as shown in A, table 2, under Years 1957, 1958, and 1960. In contrast to this, Spm was in its active phase in the cells that gave rise to the ear of each of the tillers of this plant (table 1) and it was in the active phase in the cells that gave rise to many of the ears in the Spm carrying progeny of tillers-1 and -2, as shown in B and C of table 2.

In B, table 2, under subheading Year 1957, culture 7306, is shown the phase of activity of Spm in the cells that produced ears on eight Spm carrying plants, ^{plant was} each derived from a kernel on the ear of tiller-1 of plant 7109B-1 that showed spots of pigment in a colorless background. The plants in A of culture 7306 grew from kernels that had many small spots of pigment in a colorless background. The two plants in B of culture 7306 were derived from the two kernels on this ear that exhibited only a few specks of pigment in a colorless background. Each of the six plants in A of culture 7306 had one Spm and it was linked with wx in

five of these plants but was not linked with it in one plant. Both of the plants in B of culture 7306 had two Spm elements, one of which was Y linked with wx. In plant 7306B-1, the additional Spm was located close to a_2^{m-1} in chromosome 5. Spm was in its active phase in the cells that gave rise to 15 of the 17 tested ears of the plants in culture 7306. Testcross type-2 produced some of these ears and the phenotypes of kernels on the ears produced by this cross are entered in table 15.

In table 15 likewise are entered the kernel types appearing on the ears of six plants in culture 7560, each of which was derived from a variegated, Bt, Wx kernel on the first ear of the main stalk of plant 7306A-1. In two of the six plants, Spm was linked with Wx. The one fertile ear produced by plant 7560-2 gave no evidence among its kernels of the presence of Spm in any one of them. The phenotype of the plant, nevertheless, indicated that Spm was present in this plant. In the remaining three plants, derived from the recombinant class on the parent ear, Spm was not linked with Wx and in one plant (7560-4) two Spm elements were present, both in the active phase in the cells that produced the two fertile ears of this plant. Neither of these Spm elements was linked with Wx, however. From the locations of Spm in the plants in culture 7560, it appears that the rate of transposition of Spm

in the parent plant, 7306A-1, was much higher than encountered in plants of related cultures. The percent of the recombinant classes of kernels on the ears produced by plants in culture 7306A was high, being 18.5 and this may be a reflection of the high frequency of occurrence of premeiotic transposition of Spm that occurred in these plants.

In 1957 plants were grown under culture number 7307 from kernels on the ear of tiller-2 of plant 7109B-1 and the phase of activity of Spm having Spm in the cells giving rise to tested ears of these plants, is shown in C of table 2. The plants in A of culture 7307 were derived from Bt, wx kernels that exhibited a number of small spots of pigment in a colorless background. The plant derived from each was expected to have one Spm element in its cells. Those in B of culture 7307 were derived from the four kernels on this tiller ear that showed only a few specks of pigment in a colorless background. These plants could have more than one Spm element in them. Spm was in its active phase in the cells that gave rise to all but one of the twenty-six ears that the plants in culture 7307 produced. The six plants in A of culture 7307 each had one Spm and it was linked with wx in the five plants that were wx/wx (line 1, A, table 16). Plant B-1 had only one Spm and it also was linked with wx .

Plants 7307B-3 and B-4 each had two Spm elements, one of which was linked with wx (lines 2 and 3, A table 16). The phenotype of the Spm carrying kernels on the ears produced by plant 7307B-2 was similar to that of the kernel from which this plant arose. Only a relatively few small spots or specks of pigment in a colorless background appeared in them following testcross types 1 and 2. Spm was linked with wx in this plant (line 4, A, table 16) but the recombination frequency was greater than that given by sister plants. Also, change in phase of Spm from active to inactive during development of both plant and kernel occurred later than it did in sister plants.

Variegated kernels were selected from the second ear of the main stalk of plant 7307B-2 and grown in 1958 under culture number 7572. Again Spm in most of these plants behaved in the same manner as it had behaved in the parent plant. This was shown by the late time of occurrence of change in phase of Spm, -- from active to inactive --, as well as by the same degree of increased frequency of recombination between Spm and wx (line 9, A, table 16).

On the ear of the main stalk of plant 7307A-3 and ^{of that} of plant 7307A-5, some kernels exhibiting spots of pigment in a colorless background that were in the recombinant class (wx) were grown in 1958 under culture numbers

7561 and 7562. The phase of activity of Spm in the cells giving rise to the tested ears of the plants in these two cultures is recorded in C of table 2 under Year 1958. As indicated by the data entered in lines 5 and 6 of A, table 16, Spm in these plants was linked with Wx.

The first ear of the main stalk of plant 7561-4 was sectorial.

In the cells giving rise to eight adjacent rows of kernels, Spm was in its ~~xx~~active phase and it was linked with Wx (line 6, A, table 16). In the remaining four rows of kernels, no evidence was given of the presence of Spm in any one kernel (line 7, A, table 16). In the summer of 1960, plants were grown from v riegated, Bt, Wx kernels on this ear under A and B of culture 7777. Fully pigmented^{Bt, Wx} kernels from within that part of the ear that gave no evidence of Spm, were sown under C and D of culture 7777. Each of the sixteen plants in culture 7777 had one Spm which was linked with Wx (C, table 2, Year 1960). All plants in C and D started development with Spm in its inactive phase. Change ~~xxxxxxx~~ to the active phase was much delayed as evidenced by the appearance of these plants. A few small sectors of nonpigmented tissue were present in a few of the leaves on the main stalks of some of these plants. The tillers of these plants, however, had more such sectors. Following testcross type-2, ten ears were obtained from the plants in 7777C and D but in only ~~xxxx~~ 9 of the

1085 $\underline{a}_2^{\underline{m}-1}$ carrying kernels on these ears was evidence given of the presence of \underline{Spm} , and 5 of these 9 kernels were located within a small sector on one of these ~~ten~~ ears (ear of tiller-2 of plant 77773-2). All 9 kernels were \underline{Wx} . Testcross type-3, conducted with these plants, indicated that each had one \underline{Spm} and that it was linked with \underline{Wx} . Among 596 $\underline{a}_2^{\underline{m}-1}$, Bt kernels on these ears that received \underline{Spm} from the pollen parent, 296 showed the "1 \underline{Spm} " pattern of pigmented spots in a colorless background of which 7 were \underline{Wx} and 289 were \underline{wx} , and 300 showed only a few specks of pigment in a colorless background, the "high dose" \underline{Spm} pattern, of which 284 were \underline{Wx} and 16 were \underline{wx} . The percent recombination between \underline{Spm} and \underline{Wx} , based on the ratio of \underline{Wx} to \underline{wx} within each of these two classes of variegated kernels, is 3.6. Testcross type-2, conducted with the plants in A and B of culture 7777, in which \underline{Spm} was in its active phase in the cells that gave rise to some ears of these plants, likewise showed that \underline{Spm} was linked with \underline{Wx} in these plants and that the percent recombination was low. On sixteen ears produced by testcross type-2, there was a total of 858 $\underline{a}_2^{\underline{m}-1}$ carrying kernels in which the presence of \underline{Spm} was evident. Among them, 832 were \underline{Wx} and 26 were \underline{wx} , thus giving 2.9 percent recombination between \underline{Spm} and \underline{Wx} . This very low percent of recombination was exhibited on the ears of all plants in culture 7777 regardless of the phase of activity of \underline{Spm} in the cells that

gave rise to an ear.

Pollen collected from plant 7109B-1 was used in making a number of crosses. Plants were grown during the summer of 1957 only from kernels on ears derived from two such crosses. The behavior of Spm in the plants ~~that carried it~~ is shown in D of table 2 under Year 1957, culture numbers 7312 and 7313. The plants in culture 7312 came from kernels on an ear of a plant that was a_2^{m-1} Bt/ a_2 bt, w_x/w_x . ^{in which no Spm was present.} The a_2^{m-1} in this plant belonged to class I, and its state was one that gives rise to uniformly pale-pigmented kernels when Spm is absent or when it is present but in its inactive phase. In the presence of Spm in its active phase, no pigment appears in either plant tissues or in ^{the scutellum layer of the} the kernel until a mutation-inducing event occurs which gives rise to a higher allele of A_2 that is thereafter stable in expression in the presence of an active Spm or when Spm is absent. These mutations occur late in development of the plant tissues and also late in development of the endosperm. As a consequence, the frequency of appearance of kernels having a germinal mutation is low on the ears of plants carrying this state of a_2^{m-1} . ^{Bt, w_x} The kernels from which the plants in culture 7312 grew, exhibited the presence in each of the class I state of a_2^{m-1} , derived from the female parent, and the class II state of a_2^{m-1} , ^{derived from plant 7109B-1.} derived from plant 7109B-1, and each had Spm in it. From the appearance

of the plants in culture 7312, it was evident that each carried Spm.

However, the tested ear of plant[#] 1 and of plant[#] 3 gave no evidence of Spm on each ear.

the presence of Spm among the ~~the~~ kernels. The tested ears of plants number 4, 5, and 6, however, did show the presence of Spm in some kernels

on each ear. Plant numbers 5^{and 6} proved to be a_2^{m-1} (class I) Bt/ a_2^{m-1} (class II) Bt, Wx +/-wx Spm in constitution. This was shown^{for plant number 5} by the

phenotypes of the kernels on the one fertile ear it produced in a cross with a plant homozygous for a_2 , bt, and wx and having no Spm.

Among the 128 wx kernels on this ear, 117 were uniformly pigmented;

62 pale pigmented kernels carrying the class I state of a_2^{m-1} and 55 deeply pigmented kernels carrying the class II state of a_2^{m-1} .

The remaining 11 wx kernels were variegated. Seven of these had the

class I state^{of a_2^{m-1}} and 4 had the class II state of a_2^{m-1} . Among the 124 wx

kernels on this ear, 7 were uniformly pigmented. ^{Five} ~~Six~~ of these were

pale pigmented and 2 were deeply pigmented. The remaining 117 kernels

in the wx class were all variegated. In 68 of these, the class I state

of a_2^{m-1} was present and in 49 of them the class II state of a_2^{m-1} was

present. Linkage of Spm with wx was evident and present. ~~xxxxx 7.1~~ percent of the ^{variegated} kernels on this ear were in the

recombinant classes.

In 1958, five plants were grown under culture number 7547A ^(over-landed ear) from the variegated Wx class of kernels on the above described ear that carried the class I state of a_2^{m-1} . In addition, 3 plants were grown under culture number 7547B and C from the variegated Wx kernels that had ~~xxxxxxx~~ the class II state of a_2^{m-1} . Each plant in culture 7547 carried Spm, linked with Wx, and the phase of activity of this Spm in the cells giving rise to the tested ears of these plants is given in D of table 2. It will be noted that only in 5 of the 14 ears obtained from plants in culture 7547 was Spm fully active in the cells that produced these ears. The phenotypes of the kernels on these ears are given in A of table 17. In 5 other ears, the presence of Spm was exhibited in only some of the kernels on each ear, as shown in B of table 17.

The ~~first~~ ^{second} ear of the main stalk of plant 7547A-1 was used in a cross with a plant homozygous for a_2 , bt, and wx, and carrying one Spm in its active phase (plant number 7538-3). ^{derived from the self-pollinated ear of plant 7308D-2} The phenotypes of the kernels on this ear are entered in C of table 17. Among the a_2^{m-1} (class I) carrying kernels, ~~xxxx~~ 70 were uniformly pale pigmented of which 37 were Wx and 33 were wx. Among the 70 kernels that showed ~~xxxxxxxxxxxx~~ the presence of Spm in them, 38 were Wx and 32 were wx. However, the pattern of pigmented areas in a colorless background in most of the Wx

kernels different from that of most of the wx kernels. In the wx class, there were a number of deeply pigmented spots representing mutations to A_2 and they were rather uniformly distributed over the aleurone layer. In a few of these kernels, ~~xxxxxxxxxx~~ a very small pale spot was ~~xxxxxxxxxx~~ also present. In the majority of wx kernels, in addition to deeply pigmented spots arising from mutation, there were also many areas exhibiting the pale pigmented phenotype in none of which was there a deeply pigmented spot. Small colorless areas appeared in ~~xxxxx~~ of these larger pale areas and in a few of these latter colorless areas, a small speck of deep pigment was observed. This is the ~~xxxxxxxxxx~~ phenotype that ~~would be~~ expected to appear if the majority of wx kernels have only the one β pm in them, that ^{which} was delivered by the male parent. The pale areas with colorless spots within them would represent the alternating phases of activity of β pm during development of the kernel, from ^{the} active phase at the initiation of kernel development, to the inactive phase in a cell early in endosperm development, and ^{then} a return to the active phase in a descendent cell later in development of the endosperm. The phenotype exhibited by the majority of kernels in the wx class is one that appears when three active β pm elements are present in the endosperm. Few if any pale pigmented spots would be made evident in them, ^{as they would be so} ~~those that would~~ appear would be ^{and so just in color.} small, that detection of them would be difficult.